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TITLE: Novel Oncogenes in Breast Cancer Development

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FOREWORD

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X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

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N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

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RE: Annual Summary Report for Award No. DAMD 17-99-1-9331

TITLE: Novel Oncogenes in Breast Cancer Development

A) Description of the Training:

The training to date in the laboratory of Dr. Channing Der at UNC/Chapel Hill has been excellent. During the past year I have tried to increase my knowledge of clinical aspects of cancer by attending medical grand round discussions that relate to cancer at UNC. In addition, the Lineberger Cancer Center holds weekly seminars with talks relevant to cancer biology from outside speakers which I attended. In the laboratory, I attended weekly lab meetings in which members of the lab summarized their work to date. We also hold biweekly journal club meetings primarily on the area of signal transduction and cancer biology. Within UNC, I have started collaborations with two faculty members; Dr. Carolyn Sartor and Dr. Beverly Mitchell. Last summer I attended a Keystone symposia on "Oncogene Networks in Signal Transduction", last fall I attended an AACR meeting on "Molecular Targets and Cancer Therapeutics", and this summer I attended a FASEB meeting on "The Ras Superfamily of Small GTP-Binding Proteins. I presented posters at all three meetings.

B) Description of Scientific Accomplishments:

The overall goal of this grant is to isolate novel genes involved with breast cancer development and to determine if these genes are commonly aberrantly expressed in breast tumors. I believe that this approach will lead to the successful identification of new genetic markers for breast cancer and identify novel targets for the rational design of anti-cancer drugs against breast cancer. During year one, I have focused on the goal of **Specific Aim 1**: **Identification and isolation of genes that promote the aberrant growth of breast epithelial cells.** In my first year, I obtained 8 human breast cancer tumor tissue samples; 4 noninvasive, and 4 invasive-type cancers. I successfully isolated

mRNA and generated enough cDNA from 4 of them for generation of pCTV3 retrovirus cDNA expression libraries. With one library, derived from an invasive breast cancer, I first characterized for the frequency and size of inserted cDNA and then used it to infect NIH 3T3 mouse fibroblasts for the detection of focus-formation. I have isolated, using cloning cylinders, over 30 foci of transformed cells. These cultures clearly retain a transformed growth phenotype after subcloning, a characteristic that makes it unlikely that they represent spontaneously-arising background foci. However, since some spontaneous foci of transformed cells were also seen on empty vector infected cells, it is likely that some of the foci generated from the breast cancer screen will be false positives. I have expanded these cell isolates and they have been stored in liquid nitrogen. I have encountered some technical difficulties regarding the isolation of cDNA sequences from transformed cells. In addition to these analyses, I have also performed studies to establish other cell systems for screening and refined the technology for isolation of transforming cDNAs.

Our initial screens were done with NIH 3T3 rodent fibroblasts. Advantages of using these cells are their sensitivity to transformation by a wide variety of functionally diverse oncoproteins and their ease of infection by retroviruses. However, a potentially significant limitation of these cells is their tendency towards spontaneous transformation. Another potential limitation of using NIH 3T3 cells for these screens is that fibroblasts may not be susceptible to transformation by genes important for carcinoma development. Therefore, some of my work during the past year has involved the development of epithelial cell lines for our screens. I initially analyzed two mouse mammary epithelial cell lines for oncogene sensitivity. I used C127 and NMuMG mouse cell lines and evaluated their sensitivity to a variety of oncogenes. However, each cell line suffered from a significant spontaneous transformation background problem. Therefore, I have decided not to pursue the use of these cell lines for the screens.

I have found that another non-mammary epithelial cell line, the RIE-1 rat intestinal epithelial cell line, lacks any spontaneous transforming activity, and hence appears to give us a solid screen for our libraries. I determined that RIE-1 cells can be transformed in one hit by activated tyrosine kinases (e.g., Src) or GTPases (Ras), but not serine/threonine kinases (e.g., Raf). When our lab used RIE-1 cells in a separate screening of cDNA libraries generated from a patient-derived AML or a human head and neck carcinoma cell line, 8 different transformed foci from the AML library and 4 distinct transformed foci with the head and neck library were isolated. No background foci were seen on parallel cultures infected with the empty retrovirus. Therefore, while a mammary-derived epithelial cell line would be more appropriate for our screens, I feel that the use of any epithelial cell type would be advantageous over using fibroblasts.

I am however also assessing and developing human MCF-10A breast epithelial cells for my screening since I would prefer to use breast over a colon epithelial cell system. However, I decided to avoid the use of amphotropic viruses for my screening analyses using these cells. Therefore, one aspect of my ongoing work has been to generate MCF-10A human breast epithelial cells that harbor and express exogenously

introduced ecotropic receptor. This will allow me to use ecotropic viruses for my screens. This is advantageous for two key reasons. First, the ecotropic packaging cell lines result in the production of infectious virus at higher titers. Higher titers allow me to screen a smaller number of cells. Second, ecotropic viruses can infect only rodent cells, hence, there will be minimal biohazard concerns with these library analyses. To date, the MCF-10A cell lines infected with a retrovirus encoding the ecotropic receptor has not greatly enhanced the infection rate with ecotropic virus. The logical explanation for this is the lack of high expression of the receptor. Thus, the next step in this process will be to isolate individual subclones and assay each for infection efficiency.

During the past year, I have experienced some technical difficulties in the isolation of the transforming cDNAs from the transformed cells. I have had difficulty in using PCR-mediated amplification to isolate the cDNA sequences and in some cases where the cDNAs were isolated successfully, no transforming activity was detected in subsequent transformation assays. From the transformed cell populations isolated from the screen using NIH 3T3 cells, I have failed to efficiently isolate the transforming cDNAs from a majority of the isolates. One possible concern has been the quality of the genomic DNA that was isolated from the cells for PCR-mediated amplification using DNA primers corresponding to the flanking vector sequences. Therefore, I have tried a variety of different methods to isolate the genomic DNA. To date, this approach has not effectively overcome this problem. Another possible concern is with the DNA primers. Therefore, I have tried to alter the sequences of the primers to address this concern. However, when I tried a mock situation, where NIH 3T3 cells were transformed with a retrovirus harboring an activated H-Ras cDNA sequence, these primers did effectively result in the isolation of the cDNA. Thus, for now, I have placed the isolates from the NIH 3T3 screens aside until I resolve the problem.

The second limitation I have seen is that when the cDNAs were isolated, a large percentage of them failed to show transforming activity when reintroduced into cells. I believe that this problem is associated with the likelihood that multiple retroviruses are infecting each cell. Therefore, in addition to the transforming cDNA, other nontransforming cDNAs are also likely to be isolated by PCR amplification. Thus, I need to go back to these cells and isolate different sized fragments from the PCR reaction in hopes that they might represent the correct transforming cDNA. However, I should emphasize that this effort is only worthwhile if the rate of false positives in my screens is very low. Otherwise, the other explanation for the lack of transforming activity associated with my isolates is simply that these are spontaneously transformed cells, independent of any introduced cDNA. As described above, background is certainly a problem with NIH 3T3 cells; that is why I have shifted my analyses to RIE-1 and MCF10A cells.